RecO Is Essential for DNA Damage Repair in *Deinococcus radiodurans*[▽]

Guangzhi Xu,^{1†} Liangyan Wang,^{1,2†} Huan Chen,^{1,2,3} Huiming Lu,¹ Nanjiao Ying,¹
 Bing Tian,¹ and Yuejin Hua^{1*}

Key Laboratory of Chinese Ministry of Agriculture and Zhejiang Province for Nuclear-Agricultural Sciences, Institute of Nuclear-Agricultural Sciences, Zhejiang University, Hangzhou 310029, China¹; College of Life Sciences, Zhejiang University, Hangzhou 310029, China²; and James D. Watson Institute of Genome Sciences, Zhejiang University, Hangzhou 310008, China³

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Here we present direct evidence for the vital role of RecO in *Deinococcus radiodurans*'s radioresistance. A *recO* null mutant was constructed using a deletion replacement method. The mutant exhibited a growth defect and extreme sensitivity to irradiation with gamma rays and UV light. These results suggest that DNA repair in this organism occurs mainly via the RecF pathway.

Deinococcus radiodurans is best known for its astonishing ability to resist the lethal effects of many DNA-damaging agents, including ionizing radiation, UV, hydrogen peroxide, and desiccation (1, 2, 24, 32). This striking feature is due to its extraordinary ability for DNA repair: it can reconstruct a functional chromosome from hundreds of shattered DNA fragments within hours (24). It is believed that RecA-dependent homologous recombination DNA repair plays a vital role in the genome restitution of this organism (3), although DNA repair via RecA-independent pathways is also reported to take part in genome restitution (5, 17, 26, 29, 34).

Escherichia coli and many other bacteria possess two RecA-dependent recombinational systems: the RecBCD and RecF pathways (18). These two pathways normally operate independently of each other and have different functions in vivo: RecBCD is the predominant recombination pathway and is responsible for double-strand break repair, while the RecF pathway is involved mainly in the repair of single-stranded gaps. Mutation in the *recBC* genes results in the sensitivity of *E. coli* cells to various DNA-damaging agents, unless the cells acquire mutations in the *sbcA*, *sbcB*, and *sbcC/sbcD* genes. The RecF pathway becomes active in this situation. Whereas the RecF pathway is encoded more frequently in the bacterial genome, RecBCD is absent from many bacteria (28). *D. radiodurans* is one of them: orthologous genes of neither *recBC* nor *addAB* (*recBCD* orthologues in *Bacillus subtilis*) are present in its genome (22, 33), suggesting that the RecBCD pathway is absent in this organism. In contrast, the genes coding for the RecF pathway, such as *recA*, *recF*, *recJ*, *recN*, *recO*, *recQ*, *recR*, *ssb*, *ruvA*, *ruvB*, and *ruvC*, are present in the *D. radiodurans* genome. Moreover, the *sbcA* and *sbcB* genes are absent. The expression of *E. coli* exonuclease I (25) or *recBC* (11) in *D. radiodurans* causes the cells to be more sensitive to gamma

radiation. Presumably, the RecF pathway plays a substantial role in the reconstitution of the *D. radiodurans* genome and DNA repair. Genetic studies have confirmed the importance of several RecF pathway genes in *D. radiodurans*. The deletion of *recN* (6) and *recQ* (10) and the mutation of *ruvB* (14) result in the increased radiosensitivity of the mutant bacteria. The functions and structures of the pathway proteins RecA (12, 13, 27), RecF (16), RecO (21, 23, 31), RecR (19, 20, 31), and RecQ (9) were also investigated. However, little in vivo evidence has been presented to date for the roles of the unique genes *recF*, *recO*, and *recR* of the RecF pathway in *D. radiodurans*'s radioresistance. Only a *recR* (15) partial deletion mutant, with a 27-amino-acid substitution at the C-terminal region, was reported to be extremely sensitive to cross-linking reagents like mitomycin C but remains resistant to ionizing radiation. Considering that it is a partial deletion mutant, it is possible that this *recR* mutant maintains some degree of activity. The roles of the RecF pathway in *D. radiodurans*'s radioresistance still need to be addressed experimentally. Here, we

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant marker	Reference or resource
Strains		
<i>E. coli</i> DH5α	<i>hsdR17 recA1 endA1 lacZΔM15</i>	Invitrogen
<i>D. radiodurans</i>		
R1	ATCC 13939	
MOA	As R1 but <i>recO::aadA</i>	This study
MOA-D	As MOA <i>recO::aadA</i> (pDO <i>recO_{pr}⁺</i>)	This study
MOA-E	As MOA <i>recO::aadA</i> (pEO <i>recO_{Ec}⁺</i>)	This study
TNK106	As R1 but <i>recA::cam</i>	29
Plasmids		
pMD18-T	TA cloning vector	Takara
pRADK	<i>E. coli-D. radiodurans</i> shuttle vector carrying <i>D. radiodurans groEL</i> promoter	9
pEO	pRADK derivative expressing <i>D. radiodurans recO</i>	This study
pDO	pRADK derivative expressing <i>E. coli recO</i>	This study

* Corresponding author. Mailing address: Institute of Nuclear-Agricultural Sciences, Zhejiang University, Hangzhou 310029, China. Phone and fax: 86-571-86971703. E-mail: yjhua@zju.edu.cn.

† Guangzhi Xu and Liangyan Wang contributed equally to this work.

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TABLE 2. Primers used in this study

Primer	Sequence (5'–3') ^a
MO1.....	MGATTCAATCGGAATTTGTCTTACT
MO2.....	CGCGGATCCGACGTGAGTCTAGAGAAAGA
MO3.....	CCCAAGCTTAGCATTTGACAAAAGAATGG
MO4.....	GCACGCGGGCCGAAATCATCCGTTT
MO5.....	GGAAGCGAGTCACAGACCCTC
MO6.....	GGATTTGAGGTCTGCCAGTTGA
DOF.....	CACCATATGCGCTCACGCACCGCCA
DOR.....	GAGGGATCCTGCCGTGAAGAAGTCCAAG
EOF.....	ACTCATATGGAAGGCTGGCAGCGCGCAT
EOR.....	TTCACGTAAATGCACGGTAAT

^a Underlining indicates restriction sites.

have successfully constructed a *recO* null mutant and the mutant phenotypes have been characterized.

Construction of a *D. radiodurans* *recO* null mutant. The bacterial strains and plasmids used in this study are shown in Table 1, and the primers used in this study are shown in Table 2. Disruption of the *D. radiodurans* *recO* gene (*recO_{Dr}*) was performed using a deletion replacement method as described previously (9). The construction strategy for *recO::aadA* is shown in Fig. 1A. The streptomycin resistance gene (*kat-aadA*) was obtained from the p*kat-aadA* plasmid (30), which was digested with HindIII and BamHI. A 543-bp DNA fragment corresponding to the immediate upstream portion of

the *dr0819* (*recO_{Dr}*) initial codon was amplified using primers MO1 and MO2 containing a BamHI restriction site, and a 629-bp DNA fragment corresponding to the immediate downstream region of the *dr0819* (*recO_{Dr}*) stop codon was amplified using primers MO3 (containing a HindIII restriction site) and MO4. The PCR products were digested with BamHI or HindIII and then ligated to a *kat-aadA* cassette. The tripartite ligation products were amplified using MO1 and MO4, and the resulting fragments were transformed into *D. radiodurans* R1 as described previously (8). The *recO::aadA* mutant strain was selected on TGY (0.5% tryptone, 0.1% glucose, 0.3% yeast extract) agar containing 8 µg/ml streptomycin. Mutants were confirmed by PCR using primers MO5 and MO6 and sequenced. A 998-bp DNA fragment corresponding to intact *dr0819* was the only product produced when the wild-type strain R1 chromosomal DNA was used as the template. In contrast, an approximately 1,181-bp product was obtained using genomic DNA isolated from streptomycin-resistant colonies. The 998-bp DNA fragment corresponding to intact *dr0819* was not observed in streptomycin-resistant colonies, indicating that the strain was homozygous for the *aadA* gene, displacing *recO* in streptomycin-resistant colonies (Fig. 1B). The resulting *recO* null mutant was designated MOA.

Disruption of the *recO* gene of *D. radiodurans* results in a growth defect. The influence of *recO* disruption on the growth rate was evaluated. The wild-type strain R1, the *recO* mutant

FIG. 1. *recO* mutant construction and verification by PCR. (A) Schematic of the construction of the *recO* deletion mutation. The map of *recO* in the *D. radiodurans* chromosome (top) and *recO* after disruption by its replacement with a streptomycin resistance cassette (bottom) are shown. (B) Ethidium bromide-stained agarose gel illustrating that the mutant carries a homozygous deletion of *dr0819::aadA*. Lane 1, R1; lane 2, MOA; lane 3, DNA marker.

FIG. 2. Growth curves of wild-type R1 (triangles), TNK106 (*recA* mutant) (squares), and MOA (circles). The optical densities at 600 nm (OD_{600}) are the means $\pm$ standard deviations from four independent experiments.

MOA, and the *recA* mutant TNK106 (7) cells were grown overnight (with streptomycin for MOA and with chloramphenicol for TNK106). Next, 500- μ l cultures were diluted into 100 ml TGY broth without antibiotics and shaken at 30°C. Cell

growth was monitored using a spectrophotometer at 3-h intervals. As shown in Fig. 2, MOA exhibited a severe growth defect compared with the growth of its parent strain, R1; however, the growth rate of MOA was similar to that of TNK106, a *recA* mutant, indicating that *recO* is essential for cell viability, just as *recA* is.

Disruption of the *recO* gene of *D. radiodurans* sensitizes this strain to DNA-damaging agents. Since RecO plays an important role in DNA repair, the influence of a *recO* mutation on the sensitivity of *D. radiodurans* to UV and gamma radiation was investigated. Cells for each assay were grown in TGY broth with the appropriate antibiotics to exponential phase (optical density at 600 nm, 0.4 to 0.6). For gamma irradiation, cultures were diluted to an appropriate concentration in 1.5-ml tubes and irradiated on ice with different doses for 1 h. For UV exposure, appropriate cells were plated onto TGY agar. After the medium was completely absorbed, the plates were exposed to UV at the dose rate of 1.0 J/cm²/s. Irradiated and mock-irradiated cultures were diluted to appropriate concentrations and spread onto TGY agar plates without antibiotics. Colonies were counted after incubation at 30°C for 3 to 4 days.

Disruption of *recO* resulted in a dramatic sensitivity to UV

FIG. 3. Survival curves for R1 (triangles), TNK106 (squares), and MOA (circles) and its complementary strains MOA-D (inverted triangles) and MOA-E (diamonds) following exposure to UV (A and C) and gamma (B and D) radiation. The values are the means $\pm$ standard deviations from four independent experiments.

and gamma radiation, which was of the same order of magnitude as that observed for the *recA* mutant (Fig. 3A and B). A dose of 50 J/m² of UV radiation or 200 Gy gamma radiation was sufficient to kill 90% of MOA cells, indicating that *recO* is essential for UV and gamma radiation resistance.

RecO_{Ec} only partially restores MOA gamma radiation resistance. In an effort to identify the gene responsible for the sensitivity to DNA-damaging reagents as a result of the disruption of strain MOA, a complementation plasmid with a complete *recO* gene from *D. radiodurans* or *E. coli* (*recO_{Ec}*) was introduced into the disruptant. The clones capable of restoring the disruptant's ability to resist UV and gamma irradiation damage were screened.

The complete *D. radiodurans recO* coding sequence was amplified from the genomic DNA of wild-type strain R1 using primers DOF and DOR. The fragment was inserted into the pRADK plasmid, which was digested with the same enzymes. The newly constructed plasmid was designated pDO. The *E. coli recO* complementary plasmid pEO was constructed like pDO was, except that the gene was amplified using primers EOF and EOR and that *E. coli* DH5 α genomic DNA was used as the template. The resulting complementary plasmids were confirmed by sequencing. The plasmids were transformed into MOA, and the constructs were selected on TGY agar containing 8 μ g/ml streptomycin and 3 μ g/ml chloramphenicol. The resulting constructs were designated MOA-D (complementation with pDO) and MOA-E (complementation with pEO).

The sensitivities of these strains to UV and gamma irradiation were then examined. As shown in Fig. 3C and D, the MOA-D strain was as resistant to UV and gamma irradiation as wild-type R1, a further confirmation that the sensitivity phenotype of MOA was caused by the disruption of *recO* but not by the polar effect of gene replacement, which might affect the expression of upstream and/or downstream genes. The pEO construct fully restored MOA's UV resistance, while it only partially compensated the gamma radiation sensitivity phenotype of the mutant strain. Two possible explanations exist: (i) the expression of *recO_{Ec}* did not occur in *D. radiodurans*, and (ii) RecO_{Dr} has species-specific features. Since *recO_{Ec}* can fully restore MOA's UV resistance, we consider the first possibility unlikely. RecO_{Dr} and RecO_{Ec} show low homology at the sequence level (4), and RecO_{Dr} possesses a Zn domain within the C terminus which is absent from RecO_{Ec}. Biochemical studies revealed (21) that the Zn domain of RecO_{Dr} modulates its DNA binding activity. Furthermore, gamma radiation generates more types of DNA lesions than UV radiation (4). We hypothesize that some DNA lesions, those only generated in response to ionizing radiation, may require the species-specific features of *recO_{Dr}*. However, further experiments are needed to confirm the species-specific features of RecO_{Dr}.

Here we reported the first evidence of the vital role of *recO* in *D. radiodurans*'s radioresistance. A *D. radiodurans recO* null mutant was constructed, and it demonstrated *recA* mutant-like phenotypes: slow growth and extreme sensitivity to gamma and UV radiation. This work provides useful insight into the DNA repair mechanisms exhibited by *D. radiodurans*.

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